

### REMARKS

Claims 1 and 3 – 14 are pending in the present application. Independent Claims 1, 13, and 14 have been amended herein in view of the Board's observation that the preamble of the claims is not limiting. In particular, Claims 1, 13, and 14 have been amended to relocate the text of the preamble of each claim to the body of each claim. As such, for purposes of patentability, the claimed compositions should be interpreted as being limited to those which are artificially produced canine milk compositions. As the Board has observed, this distinction may affect the treatment of the prior art against the claimed subject matter. Applicant's remarks herein below are, in large part, directed to this observation in view of the revised scope of the amended claims.

Applicant acknowledges that the Board has reversed the individual rejections based on Kakade and Oftedal; Oftedal, Kakade, and Gil; Kakade, Oftedal, Gil, and Traitler; Kakade, Oftedal, and Kinumaki; Kakade, Oftedal, and Fujimori.

The Board's new rejection of the claims under 35 U.S.C. § 102(b) and 103(a), as well as remarks regarding the Meyer Reference, are addressed as follows:

*The Rejection of Claims 1, 3 – 5, 7 – 9, and 11 – 12 Under 35 U.S.C. § 102(b), Alternatively  
Under 35 U.S.C. § 103(a)*

The Board has set forth a new ground of rejection of Claims 1, 3 – 5, 7 – 9, and 11 – 12 under 35 U.S.C. § 102(b) as anticipated and, alternatively under 35 U.S.C. § 103(a) as having been obvious, in view of disclosure regarding composition of natural beagle milk in Applicant's specification. The Board has further rejected Claims 7, 8, 9, 11, and 12; 6 and 14; and 10 and 13 under 35 U.S.C. § 103(a) as having been obvious.

Applicant has taken guidance from the Board in amending independent Claims 1, 13, and 14 herein. In particular, Applicant has amended the preamble of each of these claims to delete reference to an artificially produced canine milk substitute and to re-locate this text in the body of the claim, thereby limiting the claim from a perspective of patentability to those compositions which are indeed artificially produced canine milk substitutes.

As such, relevant to rejected independent Claim 1 and dependent Claims 3 – 5, 7 – 9, and 11 – 12 based on 35 U.S.C. § 102(b), natural beagle milk cannot be anticipatory prior art. The Board's rejection on this basis should therefore be withdrawn.

Moreover, Claims 1, 3 – 5, 7 – 9, and 11 – 12 would not have been obvious in view of natural beagle milk. As has been stated in the present specification, commercial canine milk replacers have been previously formulated based upon limited research data. Due to such limited research data, those which are ordinarily skilled in the art have previously been unable to successfully formulate a milk replacer which meets the nutritional needs of the suckling puppy. As such, natural beagle milk, the contents of which having been previously unknown, could not have obviated the present invention. Rather, but for Applicant's independent research leading to the present invention, the contents of such milk may have still been unknown. Respectfully, therefore, it is impermissible hindsight to state that natural beagle milk could obviate an artificially produced canine milk replacer, particularly since the natural beagle milk had not previously been thoroughly characterized.

The art previously cited in the prosecution history of this application is illustrative to this point. If it would have been obvious formulate a milk replacer based on natural milk, why would the prior art have deviated from what Applicant has characterized and developed? Indeed, one of ordinary skill would not have been motivated to arrive at the present invention, given the previously unknown characteristics of natural beagle milk. As a result of extensive effort and experience, Applicant has now disclosed pages of characterization of various nutrient other other material profiles related to natural canine milk. This was not information that would have been obvious to any person of ordinary skill.

Moreover, what canine milk would one of ordinary skill have used to base a commercial canine milk substitute? As described in Applicant's specification, milk composition changes throughout the course of lactation. It would not have been obvious to select a specific canine milk, selected from a specific period of lactation, upon which to conduct research. Again, to state otherwise would be use of impermissible hindsight in view of Applicant's own disclosure as set forth in the present specification. See Specification, page 5, lines 14 – 31.

In view of the foregoing, the rejections of Claims 1, 3 – 5, 7 – 9, and 11 – 12 should be withdrawn as being based upon the results of Applicant's own work as set forth in the present specification. As previously mentioned, the independent claims herein have been amended to recite, pursuant to the body of the claim, *artificially produced canine milk substitutes*. Due to this amendment, natural beagle milk, which may have otherwise anticipated the claim, cannot now anticipate the claim. Moreover, obviousness cannot be based upon the inherent features of natural beagle milk. Rather, it is Applicant who has conducted the research to determine these features, upon which

research the present invention is based. Applicant respectfully requests the withdrawal of the rejection of these claims based on natural beagle milk.

Claims 6 and 14 have been further rejected based on the combination of natural beagle milk and the teachings of Gil. For the same reasons that Claims 1, 3 – 5, 7 – 9, and 11 – 12 would not have been obvious in view of natural beagle milk, Claims 6 and 14 would not have been obvious.

Claims 10 and 13 have been further rejected in view of the combination of natural beagle milk and the teachings of Fujimori. Respectfully, it is difficult to comprehend this rejection since the rejection is based on the argument that it would have been obvious to formulate a canine milk substitute by mimicking natural beagle milk (which argument assumes, improperly, that the formula of natural beagle milk would have been readily known or determined), yet the combination of Fujimori would have actually resulted in a substitute that is not similar to natural beagle milk at all due to the use of FOS which is not detected in natural beagle milk pursuant to Applicant's research. Moreover, Fujimori does not even disclose a canine milk substitute, but is rather directed to the use of FOS in pet foods for adult cats and dogs.

For all of the above reasons, the rejections of Claims 1 and 3 – 14 should be promptly withdrawn, and the claims should be allowed. The foregoing rejections are based on improper hindsight using Applicant's own research results and disclosure.

#### *The Meyer Reference*

In view of the Board's observation regarding EP 0,259,713 (the "Meyer Reference"), Applicant has obtained an English translation of the reference which is believed to be accurate. This translation is submitted herewith, accompanied by a Supplemental Information Disclosure Statement.

As the Board did not frame its observations regarding the Meyer Reference as a rejection, Applicant will not, at this time, address this reference except to address certain statements made by the Board with respect to this Reference. In particular, the Board has noted that page 6, line 36 of the Meyer Reference (from the untranslated document) discloses a composition having a ratio of albumin and globulin to casein of 2.1 to 3.0 : 4.1 to 5.0. Based on this, the Board concludes that "at its range endpoint (casein:whey 5.0:2.1) the ratio is equal to about 70:30." However, Applicant respectfully asserts that this is an improper conclusion not supported by fact. Rather, as illustrated by the enclosed excerpt of Encyclopedia of Food Science, Food Technology and

Nutrition, Vol. 7, Eds. Macrae *et al.*, 1993 (particularly, page 4888) (excerpt enclosed for the convenience of the Examiner), sweet whey and acid whey have a crude protein content of 12.9% and 11.7%, respectively.<sup>1</sup> As such, it cannot be deduced, as the Board respectfully appears to have done, that two proteins present in whey can together amount to a total whey content. Therefore, Applicant asserts that page 6, line 36 of the Meyer Reference (from the untranslated document) does not disclose a casein to whey ratio of about 70:30.

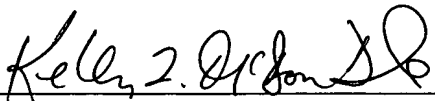
Applicant therefore respectfully requests that the Examiner consider the translation of the Meyer Reference, as suggested by the Board, in view of Applicant's foregoing remarks.

#### CONCLUSION

In light of the above remarks, it is requested that the Examiner reconsider and withdraw the new rejections posed by the Board. Early and favorable action in the case is respectfully requested.

Applicants have made an earnest effort to place their application in proper form and to distinguish the invention as now claimed from the prior art. In view of the foregoing, Applicants respectfully request reconsideration of the present application, entry of the amendments presented herein, and allowance of Claims 1 and 3 – 14.

Respectfully submitted,  
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August 20, 2003  
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<sup>1</sup> Applicant utilizes this encyclopaedic reference merely to provide perspective regarding an illustrative characterization of whey (*i.e.*, that whey is not 100% albumin and globulin). Applicant does not intend to limit whey only to the characterization provided in this particular reference.

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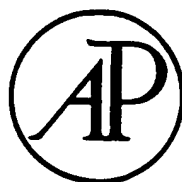
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ACADEMIC PRESS

*Harcourt Brace Jovanovich, Publishers*  
London San Diego New York  
Boston Sydney Tokyo Toronto

ACADEMIC PRESS LIMITED  
24/28 Oval Road  
LONDON NW1 7DX

United States Edition published by  
ACADEMIC PRESS INC.  
San Diego, CA 92101

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A catalogue record for this book is available from the British Library

Library of Congress Cataloging-in-Publication Data

Encyclopaedia of food science, food technology, and nutrition/edited  
by R. Macrae, R. K. Robinson, M. J. Sadler.

p. cm.

1. Food—Encyclopedias. 2. Food industry and trade—  
—Encyclopedias. 3. Nutrition—Encyclopedias. I. Macrae, R.  
II. Robinson, R. K. (Richard Kenneth). III. Sadler, M. J. (Michèle  
J.)

TX349.E47 1993  
664'.003—dc20

92-15557  
CIP

ISBN Volume 1 0-12-226851-2  
Volume 2 0-12-226852-0  
Volume 3 0-12-226853-9  
Volume 4 0-12-226854-7

Volume 5 0-12-226855-5  
Volume 6 0-12-226856-3  
Volume 7 0-12-226857-1  
Volume 8 0-12-226858-X

ISBN Set 0-12-226850-4

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# WHEY AND WHEY POWDERS

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*Protein Concentrates and Fractions*  
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*Principles of Dialysis*  
*Applications of Dialysis*

## Production and Uses

### Origins and Characteristics of Liquid Whey

Whey, the by-product of casein and cheese manufacture, was for many years treated as a waste product. Disposal was by either feeding to animals, or running to waste in streams or on to the land. In the past few decades, however, environmental pressures coupled with a recognition of the inherent value of whey solids have resulted in the development of processes for the conversion of liquid whey into a range of valuable food ingredients. This article will review the source of wheys, compositional factors affecting utilization, alternatives for processing of whey, properties of whey powders and their uses in the food industry. *See Casein and Caseinates, Uses in the Food Industry; Cheeses, Chemistry of Curd Manufacture*

### Source and Composition of Wheys

Whey may be considered as the watery substance remaining after coagulation of the casein in milk, either through the addition of acid (as in casein manufacture), or through the action of a protease such as chymosin (as in cheese manufacture). Clearly, the composition of whey will vary considerably, depending on the source of the milk and the manufacturing process involved. However, on average, whey contains about 65 g per kilogram of solids, comprising about 50 g of lactose, 6 g of protein, 6 g of ash, 2 g of nonprotein nitrogen and 0.5 g of fat. Casein wheys generally have a significantly higher level of ash than cheese wheys.

It is convenient to class whey into three major groups:

- Sweet wheys: titratable acidity 0.10–0.2%, pH typically 5.8–6.6. This category would include wheys produced from chymosin-coagulated cheeses with low levels of acidity.
- Medium acid wheys: titratable acidity 0.20–0.40%, pH typically 5.0–5.8. This class could include whey from the manufacture of fresh acid cheese such as

ricotta or cottage cheeses. *See Cheeses, Soft and Special Varieties*

- Acid wheys: titratable acidity greater than 0.40%, pH less than 5.0. This class would include casein whey made by addition of mineral acids, and some fresh acid cheese wheys.

A detailed composition of dried sweet and acid wheys is shown in Table 1.

The following points are of particular relevance in assessing options available for the processing of whey streams:

- (1) Whey has a total solids of about 6.5%, i.e. it is a fairly dilute product. Thus, to produce 1 kg of whey powder requires the removal of about twice as much water as does the production of 1 kg of milk powder. Water removal is a costly unit operation, and this factor alone mitigates against many options for whey processing.
- (2) Of the total solids in whey, more than 75% is lactose. The effective utilization of whey is therefore inextricably linked with the effective utilization of lactose. Unfortunately, lactose is not a commercially valu-

**Table 1.** Composition of sweet and acid whey powders, and whey protein concentrates

|                         | Composition (%) |               |              |         |     |      |
|-------------------------|-----------------|---------------|--------------|---------|-----|------|
|                         | Moisture        | Crude protein | True protein | Lactose | Fat | Ash  |
| Sweet whey <sup>a</sup> | 3.2             | 12.9          | —            | 74.4    | 1.1 | 8.4  |
| Acid whey <sup>a</sup>  | 3.5             | 11.7          | —            | 73.4    | 0.5 | 10.8 |
| 35% WPC <sup>b</sup>    | 4.6             | 36.2          | 29.7         | 46.5    | 2.1 | 7.8  |
| 50% WPC <sup>b</sup>    | 4.3             | 52.1          | 40.9         | 30.9    | 3.7 | 6.4  |
| 65% WPC <sup>b</sup>    | 4.2             | 63.0          | 59.4         | 21.1    | 5.6 | 3.9  |
| 80% WPC <sup>b</sup>    | 4.0             | 81.0          | 75.0         | 3.5     | 7.2 | 3.1  |

<sup>a</sup> Data from Posati LP and Orr ML (1976) *Agriculture Handbook*, No. 8-1. Washington: US Department of Agriculture.

<sup>b</sup> Data from Glover FA (1985) *Ultrafiltration and Reverse Osmosis for the Dairy Industry. Technical Bulletin*, No. 5. Reading: National Institute for Research in Dairying.

**Table 2.** Relative sweetness and solubility of lactose, sucrose and some monosaccharides

| Sugar       | Relative sweetness <sup>a</sup> | Solubility (g per 100 g solution) <sup>b</sup> |      |      |
|-------------|---------------------------------|--|------|------|
|             |                                 | 10°C   | 30°C | 50°C |
| Sucrose     | 100                             | 66   | 69   | 73   |
| Lactose     | 16                              | 13   | 20   | 30   |
| D-Galactose | 32                              | 28   | 36   | 47   |
| D-Glucose   | 74                              | 40   | 54   | 70   |
| D-Fructose  | 173                             | —  | 82   | 87   |

<sup>a</sup> Data from Pazur JH (1970). Oligosaccharides. In: Pigman W, Horton D and Herp A (eds) *The Carbohydrates: Chemistry and Biochemistry*, p 69. New York: Academic Press.

<sup>b</sup> Data from Shah NO and Nickerson TA (1978) Functional properties of hydrolyzed lactose: solubility, viscosity and humectant properties. *Journal of Food Science* 43: 1081.

able sugar, as it is not particularly soluble, nor is it particularly sweet (Table 2). These factors limit the commercial applications of whey solids very considerably. *See* Lactose

- (3) The proteins present in whey comprise about 50%  $\beta$ -lactoglobulin, 25%  $\alpha$ -lactalbumin and 25% other proteins. Whey proteins have a very high nutritional profile, are high in essential amino acids, and can have excellent functional properties. Clearly, therefore, the whey proteins are the most valuable components of whey, and most whey-processing operations (e.g. ultrafiltration, manufacture of lactalbumin) therefore aim at increasing the proportion of whey proteins in the end-product. *See* Protein, Quality
- (4) The mineral content and low pH of casein wheys severely limit their commercial exploitation. The vast majority of whey-based products are commercially manufactured from low or medium acid wheys.
- (5) Whey has a very large biochemical oxygen demand (BOD), which poses major difficulties for its disposal. It should be also noted that a number of options for whey processing, particularly those which result in an increase in the proportion of protein in the product, also result in the production of a waste product which contains most of the lactose originally present. This stream in turn will require further processing. Thus, the problems posed by the BOD of the original whey are therefore often little affected by many of the whey-processing options.

## Processing Options

Processing options for whey fall into four main areas:

- those concerned with simple removal of water (spray or roller drying to yield whey powder);
- those concerned with increasing the ratio of protein in the end-product (ultrafiltration for the manufacture of whey protein concentrates, fractionation processes for the manufacture of protein isolates, heat treatment for the production of lactalbumin); *See* Filtration of Liquids
- those concerned with utilization of the lactose in whey (treatment with lactase or heat/acid for lactose-hydrolysed products, fermentation to a range of products such as lactic acid, citric acid and single-cell protein);
- those designed to alter the mineral composition of the product (electrodialysis and ion exchange for the manufacture of demineralized products).

Each of these is considered in turn below.

## Drying of Whey

Spray drying of whey is a fairly straightforward operation, with conditions employed similar to those applying for the spray drying of milk. Thus, the whey is concentrated to 40–70% total solids and spray (or roller) dried to a moisture content of less than 5%. The drying of whey is, however, complicated by its high lactose content. Lactose exists in two isomeric forms,  $\alpha$ -lactose and  $\beta$ -lactose.  $\alpha$ -Lactose crystallizes as a hydrate, whereas solid  $\beta$ -lactose contains no water of crystallization. However, when solutions of whey are dried rapidly, there may be insufficient time for the crystallization of  $\alpha$ -lactose to the monohydrate, and it forms as amorphous  $\alpha$ -lactose. The dry lactose in the whey product is then essentially in the same form as in the liquid. Neither  $\alpha$ -lactose hydrate nor  $\beta$ -lactose is hygroscopic. However, amorphous  $\alpha$ -lactose is highly hygroscopic, and will absorb moisture from the air, resulting in a hydrate which occupies more space than the amorphous form. This effect causes the commonly observed lumping and caking in many whey powders. *See* Drying, Spray Drying

Both hygroscopic and non-hygroscopic whey powders are manufactured. The former are produced by simple drying of the whey concentrate. The manufacture of non-hygroscopic whey powders relies on the conversion of much of the lactose in the liquid concentrate to a crystalline form prior to drying. This is achieved by holding the concentrate under appropriate conditions to allow for extensive formation of  $\alpha$ -hydrate crystals. Alternatively, a process similar to instantizing may be used, in which the surface of the partly dried whey powder particles are partially humidified prior to completion of the drying operation. This stage permits additional crystallization of the  $\alpha$ -lactose during drying.



### Means for Increasing the Protein Content of the End-product

Whey solids contain about 11% protein. Many of the most popular methods of whey treatment aim at increasing this level, with end-products containing between 35% and virtually 100% protein. It should be noted that each of these methodologies results in a waste stream high in lactose, which will pose separate utilization or disposal problems.

#### *Ultrafiltration*

Ultrafiltration is the most common method used by the dairy industry to produce a range of whey products with increased protein content, known as whey protein concentrates (WPCs). Ultrafiltration relies on the passage of whey near a membrane with a pore size such that low-molecular-weight materials such as lactose and ash pass through the membrane, whereas higher-molecular-weight components such as proteins are retained. On ultrafiltration of whey, therefore, the solids content of the product retained by the ultrafiltration membrane (the retentate) is higher in protein and lower in lactose than the original whey, and the solids content of the product which passes through the membrane (the permeate) is high in lactose and ash and has minimal protein content.

WPCs are produced from a wide range of wheys, generally to protein contents of 35%, 50% and 75% (Table 1). WPC of 35% protein content is often used as a skim milk powder replacer in applications where the specific functionality of skim milk powder is not important (WPC of 35% protein content is generally significantly less expensive than skim milk powder). WPC of 50% protein content is not widely manufactured, and generally is used for specific applications only. WPC of 75% protein content can have very desirable functional properties, and these can be readily manipulated by modification of the manufacturing process. Such products often have excellent water-binding, gelation and emulsifying properties, making them sought after as functional ingredients by the food industry. *See* Emulsifiers, Organic Emulsifiers

#### *Lactalbumin Production*

Whey proteins are heat-sensitive, and can be precipitated by heat treatment under appropriate conditions of pH and ionic strength. This property is utilized in the manufacture of lactalbumin. (Note that lactalbumin – the product of heat precipitation of the proteins from whey – contains a mixture of denatured  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin and other whey proteins. 'Lactalbumin' should not be confused with  $\alpha$ -lactalbumin.) In the manufacture of lactalbumin, whey is heated to denature,

coagulate and precipitate the whey proteins; the sediment is recovered by settling and decantation (or centrifugation), washed to remove excess salt, and the product recovered by centrifugation or filtration prior to drying, grinding and bagging. The heat treatment used in the manufacture of lactalbumin results in extensive denaturation of the whey proteins, resulting in a product of poor functionality. Lactalbumin, therefore, finds its best applications in products where protein fortification is necessary, but it is not required to provide any functional properties.

#### *Protein Isolation and Fractionation*

In contrast to the precipitation of whey proteins by heat treatment in the manufacture of lactalbumin, protein isolation and fractionation methodologies aim at separation of the proteins from whey in such a form that they remain as far as possible fully undenatured, and thus retain their functionality. These products (protein concentrates and isolates) are therefore high in protein content, and can have exceptional functional properties of considerable value to the food industry.

Protein concentrates contain the whey proteins in about the same proportions as that in whey. (Note that in this article, the term 'protein concentrates' is used for high-protein products containing the individual whey proteins in about the same ratio as that present in whey, the term 'whey protein concentrate' is used for such products manufactured by ultrafiltration, and the terms 'protein isolates' and 'protein fractions' are used to refer to high-protein products with a higher ratio of a particular protein than that present in whey.) Such protein concentrates are generally manufactured by the use of a nonspecific absorbent to bind the proteins in whey, followed by elution of the proteins by treatment of the absorbent with a specific eluent. Absorbents which have been commercially used include carboxymethylcellulose and a range of mineral oxides. Although these absorbents are comparatively nonspecific, they can show preference for binding particular proteins under set conditions of pH, temperature and ionic strength. Thus, these processes can be used to produce protein isolates, for example with a higher ratio of  $\beta$ -lactoglobulin to  $\alpha$ -lactalbumin than that present in whey.

Protein fractionation technology is also developing which relies on separation of  $\alpha$ -lactalbumin from  $\beta$ -lactoglobulin on the basis of their differing solubilities under specified conditions of pH, temperature and ionic strength. It is therefore possible, for example, to separate by sedimentation most of the  $\alpha$ -lactalbumin from whey by careful manipulation of processing conditions. Both the  $\alpha$ -lactalbumin which has sedimented, and the residual soluble protein (mostly  $\beta$ -lactoglobulin) are comparatively undenatured, and thus retain their high

#### *Production and Uses*

functionality. The conditions employed in these processes are very mild, and do not result in any denaturation of the whey proteins. With such processes it is therefore possible to produce isolates high in  $\beta$ -lactoglobulin (with extremely high gel strength) and  $\alpha$ -lactalbumin (a product which may have considerable potential in nonallergenic infant foods).

### Lactose Processing Options

The options for treatment of whey involving lactose may be divided into three groups – those involving a fermentation step, those involving separation of the lactose and its utilization, and those involving enzymatic hydrolysis of the lactose to produce galactose and glucose. *See* Galactose

#### Fermentation

There are many options for the fermentation of whey described in the literature, including the production of biogas, biomass, alcohol, lactic acid and citric acid. However, the dairy industry worldwide has not taken up such opportunities to any great extent. Plants do exist for the processing of whey into biomass and alcohol, notably in the USA and New Zealand (alcohol), but the throughput of these units is comparatively small.

#### Separation of Lactose

In many respects this is a most attractive option, as it can be used also as a process for the treatment of waste streams from other whey treatment operations such as ultrafiltration. However, as indicated above, lactose is not a commercially desirable product, and the market for lactose is comparatively inelastic. Manufacture of lactose (normally  $\alpha$ -lactose hydrate) generally involves protein removal, concentration, refiltration, further concentration, induction of crystallization, and separation of crystals with a basket centrifuge.

#### Lactose Hydrolysis

The hydrolysis of lactose yields the sweet soluble sugars glucose and galactose, thus increasing the applications of the product. Such hydrolysis can be carried out by treatment of whey with lactase, or by treatment of deproteinized whey at elevated temperatures and low pH. It should be noted that it is difficult to dry hydrolysed wheys because of the tendency of the monosaccharides formed by the hydrolysis to produce glasses on the surface of the drier.

### Changes in Mineral Composition

The mineral composition of whey, particularly casein wheys, is such that it deleteriously influences the taste and applications of the product. Whole or partial

demineralization of whey is therefore a popular option with manufacturers. In general, this is accomplished by treatment of whey by ion exchange (no preferential removal of ions), or electrodialysis (preferential removal of monovalent ions). Both processes are expensive and produce high levels of intractable effluents.

More recently, an option for demineralization using 'open' reverse-osmosis membranes has been developed. These membranes allow the passage of ions and water, whilst retaining all other whey components, including lactose.

### Applications

Whey solids may be used as a food ingredient in products such as calf milk replacers, infant formulae, whey cheese, beverages, baked goods, ice cream and other dairy products, comminuted meat products and imitation milk products. In most cases, the whey solids contribute little to the functionality of the product, offering only a comparatively low-cost source of protein, carbohydrate and calcium.

Similarly, lactalbumin is used in foods where protein fortification is required, but additional functionality is not essential. Lactalbumin is commonly used in products such as baked goods and comminuted meat products.

WPCs are used where both protein fortification and functionality is required, although WPC of 35% protein content is generally employed directly as a cost-effective skim milk powder replacer. WPC of 75% protein content, with its excellent gelation properties, is often used as a cost-effective egg white replacer.

The main market for demineralized wheys is in the formulation of cow's milk-based infant formulae with a closer composition to that of human milk. Demineralized wheys also may be used effectively as beverage ingredients, where the saltiness of the undemineralized product might normally preclude its use. *See* Infant Foods, Milk Formulas

Lactose is used in sauces, instant drinks and meat products, where its low sweetness and ability to enhance flavour are advantages. Lactose is also used extensively in the confectionery and baked goods industries. Highly purified lactose is also used in the pharmaceutical industry for tablet making, and as a substrate for the manufacture of penicillin and other fermented products.

Applications of hydrolysed wheys include ingredients in foodstuffs such as beverages, and other products such as moist animal foods, where it can be used as a humectant to replace the more expensive glucose commonly used.

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## Protein Concentrates and Fractions

In the past 20 years, whey processing has been revolutionized by the development of methodologies for the manufacture of highly functional food products containing a higher proportion of protein than that of whey. Such technology generally involves either ultrafiltration, or selective separation of whey proteins (either by adsorption technology, or by utilization of the differing solubility characteristics of whey proteins under specified conditions of pH and temperature). This article will discuss the properties of the major proteins of whey, whey protein products and their methods of manufacture, and the application of such products in the food industry.

### Characteristics of Whey Proteins

Whey proteins are those which remain in solution after removal of the caseins from milk, either by treatment with chymosin, or by acidification. The distribution of proteins in skim milk is shown in Table 1. See Casein and Caseinates, Methods of Manufacture

$\beta$ -Lactoglobulin is the major protein in whey, comprising about one-half of the total protein present. It occurs in a number of variants, and has a monomer molecular weight of about 18 000. It should be noted that it is only outside the pH range 3.5-7.5 that  $\beta$ -lactoglobulin exists as a monomer. Inside this range, it generally exists as a dimer although, under certain circumstances, some variants may form an octomer.  $\beta$ -Lactoglobulin is comparatively heat-sensitive and may be denatured by heat treatment much above 60°C.  $\alpha$ -Lactalbumin has a monomer molecular weight of

**Table 1.** Average composition of warm skim milk protein

|                        | g per 100 g milk | Total protein content (%) |
|------------------------|------------------|---------------------------|
| Colloidal casein       | 2.36             | 74                        |
| Serum casein           | 0.26             | 8                         |
| $\beta$ -Lactoglobulin | 0.29             | 9                         |
| $\alpha$ -Lactalbumin  | 0.13             | 4                         |
| Bovine serum albumin   | 0.03             | 1                         |
| Total immunoglobulins  | 0.06             | 2                         |
| Other proteins         | 0.06             | 2                         |

Data from Farrell Jr HM and Thompson MP (1974) *Physical equilibria in milk: proteins*. In: Webb BH, Johnson AH and Alford JA (eds) *Fundamentals of Dairy Chemistry*, p. 465. Westport, CT: AVI Publishing.

about 14 000, and is somewhat more heat-resistant than is  $\beta$ -lactoglobulin. There are many other serum proteins (including immunoglobulins) in whey, most of which are readily heat denaturable. For example, heat treatment of skim milk at 70°C for 30 min denatures only 6% of the  $\alpha$ -lactalbumin, but 32% of the  $\beta$ -lactoglobulin and 89% of the immunoglobulins - cumulatively, such heat treatment results in denaturation of about one-third of the total serum proteins. In whey, heat treatment is often carried at a pH quite removed from that of milk. It should be noted that the pH of heat treatment of whey will have a considerable influence on the magnitude of the degree of denaturation of each of the whey proteins. See Heat Treatment, Chemical and Microbiological Changes

As globular proteins, both  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin have the potential to be highly functional food ingredients. Unfortunately, many of the processes employed for whey protein recovery result in their partial denaturation, which generally reduces the functionality of the product.

### Major Products and Applications

#### Whey Protein Concentrates

Whey protein concentrates are manufactured by the ultrafiltration of whey. In this process, whey is passed against a semipermeable membrane, which selectively allows passage of low-molecular-weight materials such as water, ions and lactose, whilst retaining higher molecular weight materials such as protein in the 'retentate'. The retentate is then further concentrated by evaporation, and spray dried to yield whey protein concentrates (WPCs). WPCs are generally available commercially with protein contents of 35, 50 and 75%. For the higher protein products, a process known as

**Table 2.** Functionality of whey proteins in food

| Functional property   | Mode of action  | Food system  |
|-----------------------|---|--|
| Solubility            | Protein solvation                                       | Beverages  |
| Water absorption      | Hydrogen bonding of water; entrapment of water          | Meat sausages, cakes, breads   |
| Viscosity             | Thickening, water binding                               | Soups, gravies, salad dressing   |
| Gelation              | Protein matrix formation and setting                    | Meats, curds, baked goods, cheese                                      |
| Cohesion-adhesion     | Protein acts as adhesive material                       | Meat sausages, baked goods, pasta products                             |
| Elasticity            | Hydrophobic bonding in gluten; disulphide links in gels | Meats, bakery products   |
| Emulsification        | Formation and stabilization of fat emulsions            | Sausages, salad dressing, coffee whitener, soup, cakes, infant formula |
| Fat absorption        | Binding of free fat                                     | Sausages, doughnuts  |
| Foaming to entrap gas | Forms stable film                                       | Chiffon, desserts, cakes, whipped toppings                             |

Data from Kinsella JE (1976) *Functional properties of proteins in foods: a survey*. *CRC Critical Reviews in Food Science and Nutrition* 7: 219.

'diafiltration' is employed, in which additional water is added to the retentate during manufacture to 'wash out' more of the low-molecular-weight materials from the retentate.

Whey proteins offer foodstuffs a wide range of potential functionality, as shown in Table 2. A number of factors influence the functional properties of WPCs. These include the source of whey, its protein content, the heat treatment applied to the whey or ultrafiltration retentate during manufacture, and the lipid content and mineral content of the WPCs.

In general, WPCs of lower protein content have more limited functionality than those of higher protein content. In many cases, WPCs serve more than one functional purpose in foods. For example, as whey proteins remain soluble over a wide pH range, and in particular near pH 4.5, they may be used in acidic drinks as protein fortifiers. They may also bring emulsifying properties to these products and, if desired, may also add turbidity. WPCs can be used as water binders in products such as baked goods and processed meats. In such cases, processing temperatures must be sufficiently high to denature the whey proteins, but not so high as to disrupt their water-binding properties. WPCs also can have excellent gelation characteristics, and can assist in the formation of heat-induced gels in meat products and baked goods. The emulsifying properties of WPCs can be employed in products such as salad dressings. WPCs may also have useful foaming properties, producing very stable foams on whipping. *See* Emulsifiers, Organic Emulsifiers; Water, Structure, Properties and Determination

The manufacture of WPCs with such widely varying and yet specific functional properties requires the careful control of manufacturing conditions. Currently, much ultrafiltration processing of whey is carried out on an *ad hoc* basis, with little understanding of the effects of individual processing parameters on the conformation

or structure of the individual proteins, or on the functionality of the product. It should also be noted that the functionality of the whey products can be permanently impaired by the use of excessive temperatures or extremes of pH during processing.

Mineral composition is an important factor determining whey protein functionality. Many of the functional properties of WPCs are considerably influenced by either demineralization or by the addition of calcium salts. Clearly, the manufacturing process employed in the whey production process will influence the mineral content of the product, and thus its functionality.

Whey proteins can also be recovered by the application of heat to whey to cause coagulation of the proteins (lactalbumin manufacture), or by adsorption or ion exchange (using adsorbents such as carboxymethylcellulose – such as in the Bi-Pro process, or inorganic oxides – such as 'Spherosil' reagents) or complex formation (with reagents such as polyphosphates). The manufacture of lactalbumin by heat treatment of whey is widespread. Some small-scale plants are commercially manufacturing whey protein isolates utilizing either Spherosil or carboxymethylcellulose. The products of these operations are highly functional, high value-added whey protein fractions which have high specific functionality. However, in general, the commercial application of these processes appears to be limited by the high costs and low protein-binding capacity of the adsorbents.

### Whey Fractions

Whey proteins can be recovered as mixtures of the proteins in whey by use of the technologies outlined above. Commercially, ultrafiltration technology is dominant for such processes. However, WPCs manufactured by ultrafiltration have not in general lived up to

their promise of offering reliable highly functional cost-effective ingredients to the food industry. In particular, the functionality of WPCs is often poor and/or highly variable. Further, the functional properties of WPCs are often well below those expected from their protein composition. This may be due to mechanical or heat damage to the proteins during manufacture, or to the presence of other compounds in whey which inhibit the development of full functionality.

To overcome such difficulties, a number of alternative options for whey protein fractionation have been developed. These aim at manufacture of protein fractions containing a higher proportion of a particular protein than that present in whey solids.

### *Ion Exchange*

Ion exchange or adsorption was briefly discussed above as a means for separation of proteins from whey. However, the adsorbents used can also selectively adsorb specific proteins from whey under specified conditions of pH and temperature. For example, under appropriate conditions certain Spherosil adsorbents can selectively remove a significant proportion of the  $\beta$ -lactoglobulin from whey, leaving a fraction rich in  $\alpha$ -lactalbumin as the effluent from the adsorption process.

Such processes generally employ batch operation, involving adsorption, elution and regeneration.

### *Ion Depletion*

This technology relies on the fact that  $\beta$ -lactoglobulin is insoluble in solutions of low ionic strength, particularly in the vicinity of its isoelectric point. This principle has been used in a number of studies which have led to pilot scale separation procedures. The products in general were substantially  $\beta$ -lactoglobulin, although other proteins were present. However, the yield of the process was poor, with only about 30% of the protein present in whey recovered as the precipitate.

### *Thermal Separation*

A series of studies have recently shown that the solubility of  $\alpha$ -lactalbumin decreases very markedly under certain conditions of pH, temperature (below that of denaturation) and ionic strength. These conditions have no effect on the solubility of  $\beta$ -lactoglobulin. Clearly, this difference in solubility characteristics may be exploited as a means of preparation of whey protein fractions. Two processes based on this principle have reached the stage of near commercialization – one in France, and the other in Australia. The major difference between the two is the starting material – the French

process uses untreated whey, the Australian process uses whey concentrated to 12% solids by ultrafiltration. In the Australian process, the pH of the ultrafiltration retentate is adjusted to 4.2, and the process of aggregation initiated by heating of the mixture to 64°C for 5 min. During this process, the  $\alpha$ -lactalbumin aggregates into small particles. The product is then diluted with water to assist in formation of larger aggregates, and the sediment (which is mostly  $\alpha$ -lactalbumin) separated, for example, by centrifugation or microfiltration. The separated sludge is evaporated and dried, to yield a fraction high in  $\alpha$ -lactalbumin ( $\alpha$  fraction). The supernatant is subjected to ultrafiltration and diafiltration (to assist in removal of ash and lactose), and dried to yield a fraction high in  $\beta$ -lactoglobulin ( $\beta$  fraction). The  $\alpha$  fraction contains about 50% protein (mostly  $\alpha$ -lactalbumin) and 40% lactose, and the  $\beta$  fraction contains about 75% protein (mostly  $\beta$ -lactoglobulin) and 15% lactose.

It is probable that the products from the French and Australian processes will be similar in functionality – in each case, the  $\beta$  fraction is low in lipid content. The  $\beta$  fraction has been shown to have excellent gelation characteristics (much greater than those shown by the best 75% WPC). Further, the gel strength exhibited by the  $\beta$  fraction can readily be manipulated by minor modification to processing conditions. Clearly, this product has considerable potential as a highly functional food ingredient, with applications similar to those of egg white.

The  $\alpha$  fraction contains most of the lipid and phospholipid in whey, and should be expected to show excellent emulsifying properties. A further application for the  $\alpha$  fraction is in 'humanized' infant foods. Although whey-based products are common components of infant foods, whey contains a significant amount of  $\beta$ -lactoglobulin, a protein which has no analogue in human milk. On the other hand, human milk does contain an analogue to bovine  $\alpha$ -lactalbumin. Clearly, therefore, infant foods based on ( $\beta$ -lactoglobulin-free)  $\alpha$  fraction may offer considerable advantages in reducing allergenic response. *See Infants, Breast- and Bottle-feeding*

### *Ferric Chloride Fractionation*

Techniques have been described in the literature for the treatment of partially demineralized whey with ferric chloride to precipitate  $\beta$ -lactoglobulin selectively as a ferric complex at near neutral pH. By contrast, at an acidic pH, almost all proteins except  $\beta$ -lactoglobulin are preferentially precipitated. In this case, the separated complex can be solubilized by a change in pH to near neutral, and the ferric ion separated by, for example, ultrafiltration. Such processes do not appear to be near commercialization.

**Table 3.** Some uses of whey proteins and whey protein concentrates in foods

|                 |                    |                  |
|-----------------|--------------------|------------------|
| Baked custard   | Coffee whitener    | Meat analogues   |
| Beverages       | Cream filling      | Meat extenders   |
| Acid-clear      | Cream icings       | Meat loaf        |
| Acid-turbid     | Cream sauces       | Meringues        |
| Neutral         | Cream desserts     | Noodles          |
| Biscuits        | Cultured beverages | Pasta            |
| Breads          | Doughnuts          | Potato flakes    |
| Cakes           | Egg white replacer | Puddings         |
| Cake fillings   | Egg yolk replacer  | Sauces           |
| Confectionery   | Gravies            | Sausage          |
| Caramels        | Hot dogs           | Sherbet          |
| Milk chocolate  | Ice cream          | Snack foods      |
| Canned beans    | Imitation cheese   | Tortillas        |
| Cereals         | Imitation milk     | Whipped toppings |
| Chocolate drink | Macaroni           | Yogurt           |

Data from Marshall KR and Harper WJ (1987) *Whey Protein Concentrates*. Bulletin of the International Dairy Federation, No. 388B, p 21. Brussels: International Dairy Federation.

### *Removal of Lipid from Whey and Whey Protein Fractions*

The lipid fraction in whey is believed to inhibit much of the potential functionality of WPCs, and whey protein fractions. The lipid fraction in whey is also partly responsible for the fouling of the membranes on ultrafiltration processing of whey. Removal of the lipid fraction can thus improve processing efficiency (if ultrafiltration is employed) as well as product functionality.

Removal of such lipid fractions from whey prior to ultrafiltration or fractionation can be achieved by microfiltration using membranes of an appropriate pore size to remove the comparatively large lipid-containing material. However, fouling of microfiltration membranes (presumably also by the lipid-containing fraction) in such processes has, as yet, mitigated against commercial adoption of this approach. It is likely, however, that improvements in microfiltration membranes coupled with appropriate adjustment of processing conditions will result in commercialization of such processes in the near future.

The addition of calcium to aggregate lipoproteins in whey has also been proposed as an alternative to microfiltration processing. Whilst this process is technically effective, it would pose particular difficulties on scaling up to commercial operation.

A number of the whey fractionation processes previously outlined result in the preferential transfer of any lipid-containing portion of the whey into one particular fraction. For example, the lipid fraction in whey is preferentially found in the  $\alpha$  fraction produced by the Australian process utilizing thermal aggregation. Such lipid material may be removed from whey fractions by,

for example, microfiltration. This would result in a protein fraction of increased functionality, and a lipid fraction with excellent emulsification characteristics.

### *Summary of Whey Fractionation Methodologies*

Of the various whey fractionation processes outlined, only the procedures based on adsorption/ion exchange involving the use of carboxymethylcellulose and Spherosil are in commercial operation, and these only in comparatively small-scale operations. The thermal procedures for whey fractionation are nearing commercialization. Of the remainder, the use of microfiltration for pretreatment of whey to remove lipid-containing material is likely to become commercial within the next few years. The remainder of the processes outlined seem unlikely to be of commercial interest.

### *Functionality of Concentrates and Individual Whey Fractions*

Of their nature, it is likely that whey protein fractions will have much greater and more reliable functionality than will WPCs, even WPCs of 75% protein content. Although the existing production of whey protein fractions is limited in the main to those from the Bi-Pro and Spherosil processes, and are of comparatively small tonnage, the ongoing development of the thermal aggregation processes will likely see production of whey fractions increase sharply in the next few years. Already whey protein fractions attract much higher returns than WPCs and, with increased production, it is probable that increased applications will be identified, resulting in increased demand. Some products in which WPCs and whey fractions have been commercially utilized are listed in Table 3.

Whey fractions and whey protein concentrates bring many valuable functional properties as food ingredients. They can modify some or all of the organoleptic, visual, hydration, surfactant, structural, textural and rheological properties of the food, resulting in improved consumer acceptance of the product. It is probable that the further development of specialized whey fractions with reliable and well-defined functional properties will see a marked increase in the application of these products over the next few years.

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